Construct	Transfection date	Transfection efficiency, by IFA, % anti-L	anti-HA	anti-V	Hrs p.e.	Media collected, hrs. p.e.	MONO LAYER, % confluency/CPE	INFECTIVITY by IFA, anti-L, i.u./ml	anti-HA	anti-V, i.u./ml & (f.f.u./ml)	IFA, Hrs. p.i.	PLAQUE ASSAY, pfu/ml (Size, mm) 24hr	65hr
1. HA+LSN	3/22/95	90-100	50-70	0	20	28 46	50/2 50/2	nt <10		nt <10	nt 18	nt <100	nt <100
2. LSN+21-1		90-100	0	0	20	28 46	60/2 40/2-3	nt <10		nt <10	nt 18	nt <100	nt <100
3. LSN+22-1		90-100	0	0	20	28 46	50/2-3 40/3	nt <10		nt <10	nt 18	nt <100	nt <100
4. LSN+21-1+19-1		90-100	0	30-40	20	28 46	50/2 40/3	nt <10		nt <10	nt 18	nt <100	nt <100
5. LSN+22-1+19-1		90-100	0	50	20	28 46	50/2-3 30/3	nt <10		nt <10	nt 18	nt <100	nt <100
6. 21-1+22-1+19-1		0	0	0	20	28 46	80/- 90/-	nt <10		nt <10	nt 18	nt <100	nt <100
7. HA+P5-1		0	100	5-10	20	28	70/-	<10	1x10^6	+(+)	18	<100	300
8. P5-1		0	0	0	20	28 46	70/- 90/-	nt <10		nt <10	nt 18	nt <100	nt <100

Note: In transfections 3/15/95 and 3/22/95 were used reduced same 1.5:1 "cap" :GTP ratios for all in vitro transcriptions. Is this the reason for low transfection efficiency of P5-1 and 19-1; 18-*; 19-1; 21-1; 22-1 ?????
20% of HA-infected cells have VEE Ag (by double IFA with a-rabbit-Rho). In these cells, HA looks weak ? due i) 1/2 synthesis or ii) cross-reactivity???

Construct	Transfection date	anti-L	anti-HA	anti-V	Hrs p.e.	Media collected	MONO LAYER	anti-L	anti-HA	anti-V	IFA Hrs. p.i.	24hr	65hr
1. HA+P5-1	3/30/95	0	100	100	20	27	40/-	<10	5x10^7	+(+)	19	5x10^4(VS)	
2. LSN+P5-1		100	0	100	20	27	40/1	5X10^6	<10	+(+)	19	3X10^4(VS)	
3. HA		0	100	0	20	27	40/-	<10	<10	<10	19	<100	
4. LSN		100	0	0	20	27	40/1	<10	<10	<10	19	<100	
5. P5-1		0	0	0	20	27	50/-	<10	<10	<10	19	<100	
6. LSN+P22-1+P19-1		100	0	30-40	20	27	40/1	<10	<10	<10	19	<100	
7. LSN+P22		100	0	0?	20	27	40/0-1	<10	<10	<10	19	<100	
8. P22-1+P19-1		0	0	0	20	27	40/-	<10	<10	<10	19	<100	

Note: For this transfection, cap:GTP ratios in in vitro transcriptions were 1.5:1 (LSN and HA); 3:1 (P5-1); 4:1 (P22-1); 3.4:1 (P19-1).

Construct	Transfection date	anti-L	anti-HA	anti-V	Hrs p.e.	Media collected	MONO LAYER	anti-L	anti-HA	anti-V	IFA Hrs. p.i.	24 hr p.i.	80 hr p.i.
1. LSN 8ul	4/10/85	75	0	0	21	27	80/+/-	<10	<10	<10	20	<100	<100
2. P5-1 6ul		0	0	0	21	27	80/+/-	<10	<10	<10	20	<100	<100
3. HA 6ul		0	100	0	21	27	80/-	<10	<10	<10	20	<100	<100
4 HA+P5-1 6+6		0	100	75-90	21	27	70/-	nt	5x10^7 1x10^8	+(few+)	20	4x10^3 S	4x10^3
5. HA+P5-1 15+10		0	100	90-100	21	27	70/-	nt	4x10^7 1x10^8	+(-)	20	<100	<10^3 dead at 10^-2
6. LSN+P5-1 8+6		75-100	0	40-60	21	27	70/1	1x10^6 5x10^6	nt	few+(-)	20	<100	<100
7. LSN+P5-1 16+10		75-100	0	50-70	21	27	70/1	4x10^6 7x10^6	nt	virus	20	6x10^3	6x10^3

Note: cap:GTP ratios 1.5:1 for HA&LSN; 3:1 for P5-1. Relative amounts of RNAs in transfectios are shown. Recombination efficacy and virus origination seems to depend on amount of RNA in transfection (comp. 6. vs 7.) or see note 4/12/95.

Monolayer of BHK in t75 flask were infected with 2 m.o.i. of 1) LSN reps (1 ml from 2/1/95) or 2) HA reps (1 ml from 2/1/95), incubtd for 10 hr for protein analysis. T25 flasks were infected with 2 m.o.i. of the same materials (0.33 ml) and incubtd for 50 hr for plaque assay, plaque picks?, rec. virus isolation, PCR ans 26s region sequencing (red pen on t25/-70). HA+P5-1 ans LSN+P5-1 showed recombinants in double IFA: cells positive for VEE were negative for HA/LSN proteins.

EXHIBIT C

Construct	Transfection date	Transfection efficiency, by IFA, % anti-L anti-HA	anti-V	Hrs p.e.	Media collected, hrs. p.e.	MONO LAYER, % confluency/CPE	INFECTIVITY by IFA, anti-L, i.u./ml anti-HA	anti-V, i.u./ml & (f.f.u./ml)	IFA, Hrs. p.i.	PLAQUE ASSAY, pfu/ml (Size, mm) 24hr	65hr
1. LSN	4/12/95	80-90	0	21	27	70/+/-	1x10^3??!	virus?!!	16	1x10^6 LMS	1x10^6
2. LSN+P21-1		80-90	0 or weak 10?		27	70/+/-	<10	<10	16	<100	<100
3. LSN+P23-2		80-90	0 or few or weak 10?		27	70/+/-	<10	<10	16	<100	<100
4. LSN+P19-1		70-80	10	21	27	70/+/-	<10	<10	16	<100	<100
5. LSN+P21-1+P19-1		50-70	30	21	27	70/+/-	<10	<10	16	<100	<100
6. LSN+P23-2+P19-1		60	20	21	27	70/+/-	<10	<10	16	<100	<100
7. P21-1		0	0	21	27	80/+/-	<10	<10	16	<100	<100
8. P23-2		0	0	21	27	80/+/-	<10	<10	16	<100	<100

Note. The origin of iu and pfu in 1. unclear (contamination?). Comp. to transfection 3/15/95, (3); and 4/10/95,(7).

4/26/95	a-LSN	a-VEE rabbit	mouseHMAF			CPE				24 hr p.i.	80 hr.p.i
1. LSN+P6-2	90	10-20	10-20	22	27	I	1x10^4	virus	19	5x10^3 S	5x10^3
2. LSN+P10-1	90	10-20	10-20	22	27	I	5x10^4	virus	19	2x10^4 MS	2x10^4
3. LSN+P16-1	60	10-20	10-20	22	27	I	2x10^3	virus	19	2x10^5 MS	2x10^5
4. LSN+P23-1	90	10-20	10-20	22	27	I	4x10^4	virus	19	3x10^4 LS	3x10^4
5. LSN	50, less DNA	0	+/-!!!	22	27	I	nt	<10	19	<100	<100
6. P6-2+P10-1	0	0	0	22	27	-	nt	<10	19	<100	<100
7. P16-1+P23-1	0	0	0	22	27	-	nt	<10	19	<100	<100

4/29/95	a-LSN	a-VEE rabbit	mouseHMAF			CPE				24 hr p.i.	65 hr
1. LSN+P5-1	100	70	70	18	25	nt	2x10^6	IU&FFU	21	3x10^3 SM	3x10^3
2. LSN+P24-3	100	70	70	18	25	nt	<10	<10	21	<100	<100
3. LSN+P21-1	100	0	0/+?	18	25	nt	<10	<10	21	<100	<100
4. LSN+P19-1	100	10	5-10	18	25	nt	<10	<10	21	<100	<100
5. LSN+P24-3+P21-1	100	60	60	18	25	nt	2-3x10^6	100-200 IU/ml	21	<100	4/29
6. LSN	100	0	0	18	25	nt	<10	<10	21	<100	<100
7. P5-1+P24-3	0	0	0	18	25	nt	<10	<10	21	<100	<100
8. P21-1+P19-1	0	0	0	18	25	nt	<10	<10	21	<100	<100

25 cm^2 of BHK was infected with 1 ml of transfection 5 (about 1 moi) 24 hr p.i.: CPE= I , PFU<100 (24&65 hr)
72 hr p.i.: CPE=III-IV; PFU<100(24

Construct	Transfection date	Transfection efficiency, by IFA, % anti-L	anti-HA	anti-V	Hrs p.e.	Media collected, hrs. p.e.	MONO LAYER, % confluency/CPE	INFECTIVITY by IFA, anti-L, i.u./ml	anti-HA	anti-V, i.u./ml & (f.f.u./ml)	IFA, Hrs. p.i.	PLAQUE ASSAY, pfu/ml (Size, mm) 24hr transfected	65hr transfected	24hr infected	65 hr infected
	5/14/95														
1. N-Rep+21-1+24-3		90		70	22	24	+/-	5x10^5	<10	<10	22	<100	nt	<100	<100
2. GPC-Rep+21-1+24-3		?		40	22	24	+/-	<10^4?	<10	<10	22	<100	<100	<100	<100
3. HA-Rep+21-1+24-3		?		70	22	24	-	<10	3x10^5	<10	22	<100	<100	<100	<100
4. N-Rep		50		0	22	24	+/-	<10	<10	<10	22	<100	<10000	<100	<100
5. GPC-Rep		?		0	22	24	+/-	<10	nt	<10	22	nt	nt	<100	<100
6. HA-Rep		0		0	22	24	-	<10	<10	<10	22	<100	<1000	<100	<100
7. 21-1+24-3		0		0	22	24	+/-	<10	nt	<10	22	<100	<100	<100	<100

25 cm^2 of BHK was infected with 1 ml of transfections 2 and 3 (about 0.1 moi) 2: 24 hr p.i.: CPE<I , PFU<100(24&65 hr)
72 hr p.i.: CPE=I; PFU

LabTek BHK infected with 0.1 ml of medium from transfection, incbted 24 hr, sups for plaque assay

3. 24 hr p.i.: CPE<1, PFU<100(24&65 hr)
72 hr p.i.: CPE=II-III; PFU

	6/19/95						
1. N+21-1+24-3		40		-	22	No	90/- (24 hr p.e)
2. GP+21-1+24-3		-		-	22		90/-
3. HA+21-1+24-3		-	40	-	22		90/-
4. N+GP		20-30		-	22		90/--/+
5. GP		-		-	22		90/--/+
6. HA		-	70	-	22		90/-
7. 21-1+24-3		-		-	22		70/-

Media were not collected because of the low transfection efficiency. BHK were grown not in CO2 incubator prior e.p., transfection was made 1 day later tnan necessary (too dense monolayer), CO2 incubator was without CO2 during cell incubation p.e.!!!!!

REPEAT

	6/25/95								
1. N-Rep+21-1+24-3		50		5-10	19	28	Nt	Less or equal 10^4	<10
2. GPC-Rep+21-1+24-3		-?		10	19	28		?	<10
3. HA-Rep+21-1+24-3		-	?	10	19	28		Less or equal 10^4	<10

Note. Transfection efficiency very low; RNA quality or amount was poor?

Small (pin point size) colonies 3 days p.i., w/o dependance on dilution of sample (come from cells?)

	7/2/95	Ls	HA	VE			27 hr p.e.	Ls	HA	VE	p.i.	24 hr p.i.	65 hr
1. N-Rep+21-1+24-3		90-100	0	90-100	22	27	I-II/50%	4x10^7	nt	100iu/ml	21	<100	<100
2. GPC-Rep+21-1+24-3		90-100	0	80/round	22	27	II/50%	?	nt	50iu/ml	21	<10!	<10
3. HA-Rep+21-1+24-3		round 0	100	90-100	22	27	0/70%	nt	4x10^7	100iu/ml	21	<100	<100
4. N-Rep		60-70	0	0	22	27	II-III/50-60%	nt	nt	<10	21	<10	<10
5. GPC-Rep		80-90	0	0	22	27	III/40%	nt	nt	<10	21	<10	<10
6. N-Rep+GPC-Rep		round 90-100	0	0	22	27	III/40%	nt	nt	<10	21	<10	<10
7. Electroporated NC cells		0	0	0	22	27	0/70-80%	nt	nt	<10	21	<100	<100

1 slide of 1.-infected BHK is fixed and kept at -20'C for GPC Mabs.

0.7 ml passed in 25sm^2 BHK 48 hr <100(24&65hr p.i.)

Construct	Transfection date	Transfection efficiency, by IFA, % anti-L	anti-HA	anti-V	Hrs p.e.	Media collected, hrs. p.e.	MONO LAYER, % confl uency/CPE	INFECTIVITY by IFA, anti-L, i.u./ml	anti-HA	anti-V, i.u./ml & (f.f.u./ml)	IFA, Hrs. p.i.	PLAQUE ASSAY, pfu/ml (Size, mm) 24hr65hr transfected	24hr65 hr infected
	7/9/95												
1. N-Rep+21-1+24-3		100	0	100	21	28	-/80%	1x10^8III	nt	200iu/ml <10ffu/ml	20	<100	
2. GPC-Rep+21-1+24-3		?	0	40	21	28	I-II/70%	?not detected	nt	200iu/ml <10ffu/ml	20	<100	
3. HA-Rep+21-1+24-3		0	100	100	21	28	-/80%	nt	6x10^7	200iu/ml <10ffu/ml	20	<100	
4. N-Rep+GPC-Rep		80	0	0	21	28	II/50%	nt	nt	<10iu/ml <10ffu/ml	20	<100	
5. N-Rep+GPC-Rep		80	0	0	21	28	II-III/40%	nt	nt	<10iu/ml <10ffu/ml	20	<100	